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Performance of fast-absorbable suture and histo-glue in closing incisions in brown trout

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Running headline: Wound closing with suture or glue

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Abstract

Telemetry has become a standard tool in fish research, but tagging methods still need refinement to achieve better results and to improve animal welfare. One of the problems reported from evaluations of surgical implants is unsatisfactory wound closure. Thus, researchers struggle to find better ways to close incisions, typically for implants of tags under field conditions. Problems are regularly encountered when closing incisions with traditional absorbable or non-absorbable suture, including decreased growth, slow wound healing, erythema and necrosis at sutures. In this study, survival, growth, tag expulsion rate and incision healing was compared among three groups of dummy transmitter-tagged wild brown trout *Salmo trutta* where incisions were closed with two types of suture material (absorbable vs. fast absorbable) and Histo-glue. The tagged fish were kept in semi-natural ponds for 20 days. Survival did not differ between groups, but growth of the tagged fish was lower than that of the control group. Histo-glue gave the best healing, but resulted in high tag loss rate (33%). The fast absorbable suture did not disappear faster than normal absorbable suture, healing and tag loss was similar. The use of fast absorbable suture may hold potential for improving the procedure and should be further tested.

Introduction

Many management decisions are being based on results from telemetry studies (Hussey et al. 2015). Results from several studies have documented that electronic tags can be implanted and carried by fish without significant effect on mortality and growth (e.g. Jepsen et al. 2002; Wargo Rub et al. 2014), but more studies of tagging effects should include focus on tagging-induced changes in behaviour (Wilson et al. 2016).

A major concern in many telemetry studies is the potential adverse effect of capture, handling, tagging and carrying a transmitter. The importance of these effects is best studied when the fish remain in their natural environment, where negative effects leading to a reduced performance may influence growth and survival (e.g. Jepsen and Aarestrup 1999; Jepsen et al. 2008a). Beside the validity of the results there is another important aspect; the ethical side of the methods, where researchers are obligated to seek refinements of techniques to achieve optimal animal welfare in accordance with the 3 R-principle (e.g. Paquet and Darimont 2010).

From the contemporary fisheries literature, incision closure techniques are likely the most studied and described, but at the same time the least understood (Wargo Rub et al. 2014). Similar to surgery conducted on warm-blooded animals, the purpose of telemetry implant surgery is to close the body wall in a way that promotes the most efficient healing. This will be achieved when the disruption of tissue is kept to a minimum, the tissue is maximally apposed, and closure materials are benign.

The available suture materials were not developed for use in aquatic environments, nor were they developed for use with poikilotherms, and therefore, they may not perform in fish as one would expect them to perform in mammals. For example, absorbable sutures have been found in fish recaptured up to 6 months after tagging (Jepsen et al. 2008a). Thus, Harms (2005) recommend removing sutures at two weeks post-op to avoid complications. However, this is not possible for

most field studies as tagged fish are typically released shortly after the implant surgery. Researchers have a choice of braided or monofilament suture, absorbable or non-absorbable suture. Many researchers have abandoned the use of braided silk and most use plain monofilament or modern absorbable material (e.g. Ivaskauskas et al. 2012; Wargo Rub et al. 2014). A comparison of silk, absorbable and non-absorbable suture material for closing incisions in rainbow trout *Oncorhynchus mykiss* demonstrated that braided silk caused more erythema than monofilament, but that healing was similar (Wagner et al. 2000). The same was observed by Ivaskauskas et al. (2012) but here braided silk also caused more expulsions of tags. Thoreau and Baras (1997) found incision healing to be faster in blue tilapia *Oreochromis aureus* sutured with monofilament than with catgut or braided silk. Cooke et al. (2003) found no significant difference in survival and wound healing between smallmouth bass sutured with non-absorbable silk or monofilament. Jepsen et al. (2008a) showed that absorbable Vicryl performed better than monofilament in a 6 months study on wild brown trout. However, in most studies, where surgical implant is used, adverse effects are observed in the time period from days to weeks post tagging. These effects can be unsatisfactory healing of incisions, which in turn can lead to reduced growth of tagged fish compared to controls. Some studies have tested the use of alternative means of incision closure, such as glue and knotless suture, but with little success (Woodley et al. 2013; Raoult et al. 2012). A consensus of optimal suture material for incision closure in fish has not been reached (Wagner et al. 2000; Cooke et al. 2003; Chapman and Park 2005; Deters et al. 2010; Boone et al. 2013). Thus, Wagner and Cooke (2005) found that 92% of surgeons preferred monofilament suture, 47% used an absorbable filament, and 27% used a non-absorbable suture, whereas few used silk, staples, glue, or a combination of suture and glue.

This study aimed at comparing the performance of two types of suture material and histo-glue on wild brown trout under semi-natural conditions. A similar test performed under controlled lab conditions would have been far easier, but we believe that working with wild fish in the wild is the best way to evaluate methods that may influence feeding, predator avoidance, competition, exposure to pathogens, temperature challenges, etc.

Material and Methods

A total of 85 wild brown trout (13-26 cm TL) were captured in a small stream by upstream electrofishing on June 9 and 10, 2015. Upon capture each fish was randomly assigned to one of four treatment groups in roughly equal numbers: Control (n = 21), Vicryl (n = 21), Safil (n = 22) or Histo-glue (n = 21). Control fish were anaesthetised in Benzocaine, measured for total length (to the nearest ± 1 mm TL) and body mass (to the nearest ± 0.1 g) and tagged individually with uniquely coded 12 mm PIT tags. These were inserted into the body cavity through a small incision (~ 2 mm) using a scalpel (no incision closing). Fish in the other three treatment groups were processed similarly to the control group, but had a dummy tag (no antenna) inserted into the body cavity aside from the PIT tag. Incisions were 10-15 mm. The epoxy resin for the dummy tags was purchased from Advanced Telemetry Systems of Isanti MN, and is identical to that used for real transmitters. The dummy tags measured 9 x 25 mm and weighed 1.4 g in air (giving tag/bm-ratios of 0.7- 4.3%, mean: 2.1%). The incisions were closed with absorbable suture (Vicryl 4-0 FS-2, Ethicon), fast absorbable suture Safil® Quick (Braun), or Histoacryl®L (Braun), herein called Safil, Vicryl and Histo-glue, respectively. Safil is a synthetic absorbable sterile surgical suture material made of polyglycolic acid, a polymer with a low molecular weight. The manufacturer informs that this suture should be partly dissolved after 7 days and fully lost after 14 days (in human tissue). Histo-glue is a cyanoacrylate compound comparable to what has been tested on fish before (eg. Raoult et

al. 2012). The surgical procedure was performed as described in Jepsen *et al.* (2008b) with a single suture to close the incisions in the Safil and Vicryl groups. Recent tests (Jepsen et al. 2013; own unpublished results) have documented that one suture is sufficient to fully close a 10-15 mm incision in most cases. Surgical implants were performed by an experienced fish surgeon.

Procedures were in accordance with guidelines from the Danish board for use of experimental animals, described in permission (2012-DY-2934-00007/BES).

After treatment and subsequent recovery fish were released into two 200 m² drainable ponds, with equal number of individuals from each treatment in each pond. Fish were captured within 300 m of the ponds and time from capture to release was 20 to 120 minutes. The ponds are shallow (1 m) with pads of vegetation and a rich macro-invertebrate fauna. The ponds are fed with a steady flow of groundwater and remained relatively cool and clear during the study period. Logging of water temperature revealed that the temperature never exceed 22°C at the outlet and was significantly cooler (8 °C) at the inlet and bottom.

After 20 days, the two ponds were drained to a very low water level and electro-fished (100 % efficiency) with a three phased 800w generator. All captured trout were euthanized and length and body mass were recorded. The next day, each fish were necropsied and a photo was taken to document healing and tissue reaction. Healing of the incisions was evaluated using an ordinal scale ranging from 0-5 (adapted from Wagner et al. 2000), zero being a perfectly healed, healthy incision, 2: healed, but with some scar tissue, 3: healed, but with inflammation and possibly suture remaining, 4: Not fully healed, gaping, some necrosis, 5: open wound with severe inflammation.

Data analysis

Generalized linear models (GLMs) with a gamma distribution and an identity link function were used to compare initial length and mass of trout in each treatment group. Pond was included as a

factor in the models to account for potential pond effects. A GLM with binomial distribution and logit link function (logistic regression) was used to compare survival among treatment groups. We also included initial length and pond in the model. Specific growth rate (SGR; g day⁻¹) for mass was calculated according to the formula:

$$\text{SGR} = (\log_e M_f - \log_e M_i) \times t^{-1}$$

where M_i and M_f are initial and final mass (g), respectively and t is the time in days. We used a GLM with a Gaussian distribution and an identity link function to model the average SGR for trout among the four treatment groups, including pond as a fixed factor. The healing score of the tagging incisions was compared among treatment groups tagged with dummy transmitters (i.e., Vicryl, Safil and Histo-glue) using chi-squared test of independence. All statistical analyses were performed in R 3.0.1 (R Development Core Team 2013). Assumption of homogeneity of variance for the GLM models was based on visual inspection of residual plots. Variation in association with recorded mean values is given as standard deviation (\pm SD) throughout. Statistical significance was assessed at a 0.05 alpha level.

Results

Survival and growth

There was no significant difference among the four treatment groups in terms of initial length (GLM; $F = 0.361$ $df = 3$, $p = 0.781$) or initial body mass (GLM; $F = 0.097$, $df = 3$, $p = 0.962$; Table 1). In addition, initial length (GLM; $F = 0.082$, $df = 1$, $p = 0.775$) and body mass (GLM; $F = 0.064$, $df = 1$, $p = 0.801$) of the trout did not differ significantly between the two experimental ponds.

A total of 65 of the 85 tagged trout (76 %) were recaptured at the termination of the study, whereas the remaining 20 fish (24 %) were lost. The probability of survival was not significantly related to treatment group (GLM; LRT = 3.227, df = 3, $p = 0.358$), initial length (GLM; LRT = 0.024, df = 1, $p = 0.878$) or pond (GLM; LRT = 0.804, df = 1, $p = 0.370$; Table 1).

The average SGR (g day^{-1}) for mass did not differ among treatment groups (GLM; $F = 1.950$, df = 3, $p = 0.131$; Figure 1) or the two ponds (GLM; $F = 0.392$, df = 1, $p = 0.533$). However, trout implanted with dummy transmitter tags (i.e., trout from the Vicryl, Safil, Histo-glue groups pooled) had marginally lower SGR than control fish (GLM; $F = 4.1148$, df = 1, $P = 0.046$).

Wound healing and tag expulsion

The majority of the recaptured fish implanted with dummy transmitters appeared to be in well-fed condition and in good health. Inspection of the incisions revealed that some were healed very nicely, while others were gaping, bulging or irritated. Of the 17 surviving fish sutured with Vicryl, 12 (70%) still had the suture in place and for the 14 recaptured fish in the Safil group, 9 (64%) still had suture. In 11 instances (5 Vicryl, 6 Safil), the suture had some degree of visible bio-growth/fouling. In several fish, where the suture was shed, the scars had the characteristic X-shape, indicating that the suture was shed through the skin, i.e., not dissolved, but “dragged out”, like non-absorbable suture. A proper dissolved suture leaves a \div shaped scar (own observations). The healing score of the tagging incision differed significantly between closure method (Chi-squared test: $\chi^2 = 28.504$, df = 10, $p = 0.001$, Fig. 2). Histo-glue resulted in the best incision healing as compared with Safil ($\chi^2 = 13.985$, df = 4, $p = 0.007$) and Vicryl ($\chi^2 = 18.936$, df = 5, $p = 0.002$). The incision healing score was not statistically different between Safil and Vicryl ($\chi^2 = 2.466$, df = 3, $p = 0.481$; Fig. 2). A total of 10 fish (16%) had lost the dummy transmitter tag after 20 days; two from the

Safil group, two from the Vicryl group and 6 from the Histo-glue group (Table 1). The mean tag/BM- ratio for the 10 fish was 2.4%, only slightly higher than the overall mean of 2.1%.

Discussion

This study demonstrated that closing tagging incisions with Histo-glue gives better healing than with sutures, but lead to high loss rate of the implanted dummy transmitters (33%). Other studies have reported similar high tag expulsion rates with glue as well as problems with skin necrosis at the glued area (Raoult et al. 2012; Jepsen et al. 2002). In the present study, the glue caused no tissue reaction. However, it appeared that the Histo-glue provided very little or no closing effect and if the incision had been left open, we would likely have seen similar results. A tag loss of 33 % in a 20 days period is not acceptable for most telemetry studies.

An earlier study indicated that the absorbable suture (Vicryl) caused fewer problems with incision healing and tag expulsion than did non absorbable suture (Ethilon) (Jepsen et al. 2008a). Closing incisions with fast absorbable suture material (such as Safil) does provide a viable alternative to Vicryl, but despite faster shedding of Safil sutures compared to Vicryl, a proportion of the fish had similar healing issues, such as irritation, erythema and necrosis. Thus, neither Safil nor Histo-glue clearly reduced the problems associated with closing incisions and at the same time provided acceptable tag retention.

When comparing all dummy transmitter-tagged fish with the control group, it appeared that surgical implants of the tags had a negative effect on the growth of trout, although mean growth was positive in all groups. The insertion of a 12 mm PIT tag in the control fish is not comparable with a surgical

implant, due to the tiny incision and the low mass of the PIT tag. The observed reduced growth did not affect survival. Reduced growth may be caused by the energetic costs associated with incision healing, by carrying the additional weight of the tag or by a changed behaviour/performance leading to reduced feeding or a combination of these. The fish appeared to be well-fed and in good health, so maybe carrying the tag as well as recovery from the surgery have led to reduced feeding or simply that the presence of the tag in the body cavity have reduced appetite. Most similar studies of surgical implants have not found reduced growth caused by tagging (e.g. Cooke et al. 2003; Bégout Anras et al. 2003; Adams et al. 1998; Martinelli et al. 1998; Jepsen and Aarestrup 1999). However, Greenstreet and Morgan (1989) found reduced growth of hatchery-reared pre-smolts tagged with relatively large external acoustic tags and Jepsen et al. (2008a) found reduced growth of tagged (surgical implants) wild trout in a natural river. Tagging effects on growth are dependent on species, tag/BM-ratio, feeding strategy and the duration of study (Wargo Rub et al. 2014). Although a slight reduction in growth is not a serious effect, it may be an indicator of altered behaviour of tagged fish, which in turn could be a problem in most telemetry studies.

The fact that 20 fish disappeared from the two experimental ponds may seem surprising, but it was obvious that predators had visited the ponds as four of the recaptured trout had clear bite-marks (mammalian). The ponds were open to all predators and both grey heron (*Ardea cinerea*) and otter (*Lutra lutra*) are regularly observed in the vicinity of the ponds. Tagged and control fish had similar survival, indicating no clear effect of tagging on predation risk, like reported from earlier studies (Jepsen et al. 2008b; Janak et al. 2012).

We used this study to compare the efficiency of three methods of wound closure and must conclude that none offers an optimal solution to the problems associated with closing of incision. Further tests of alternative wound closing methods are relevant.

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Table 1. Information of the tagged fish. Number of individuals, survival, size and tag expulsions in each treatment group.

Treatment group	No. tagged fish	No. recaptured fish (survival)	Mean initial TL (cm) \pm SD	Mean initial weight (g) \pm SD	No. tag expulsions
Control	21	16 (76%)	15.7 \pm 3.37	45.6 \pm 36.64	-
Vicryl	21	17 (81%)	16.1 \pm 3.01	46.5 \pm 29.80	2 (12%)
Safil	22	14 (64%)	16.6 \pm 3.14	49.7 \pm 28.12	2 (14%)
Histo-glue	21	18 (86%)	16.1 \pm 2.45	47.2 \pm 21.64	6 (33%)

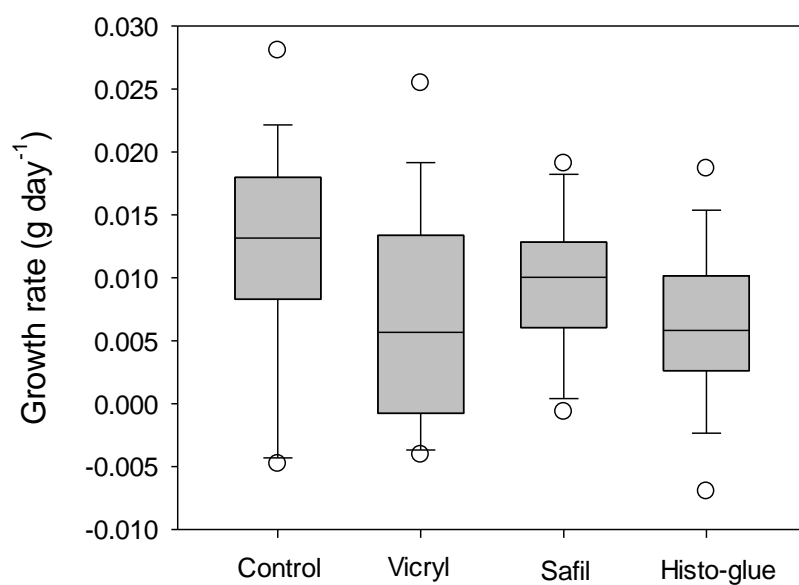


Figure 1. Boxplot showing specific growth rate for mass (g day^{-1}) of brown trout *Salmo trutta* among treatment groups. Horizontal lines within each box represent median specific growth rate of mass, ends of boxes represent the 25th and 75th percentiles, and whiskers represent the 10th and 90th percentiles. Open circles indicate outliers outside the 10th and 90th percentiles. See Table 1 for sample sizes.

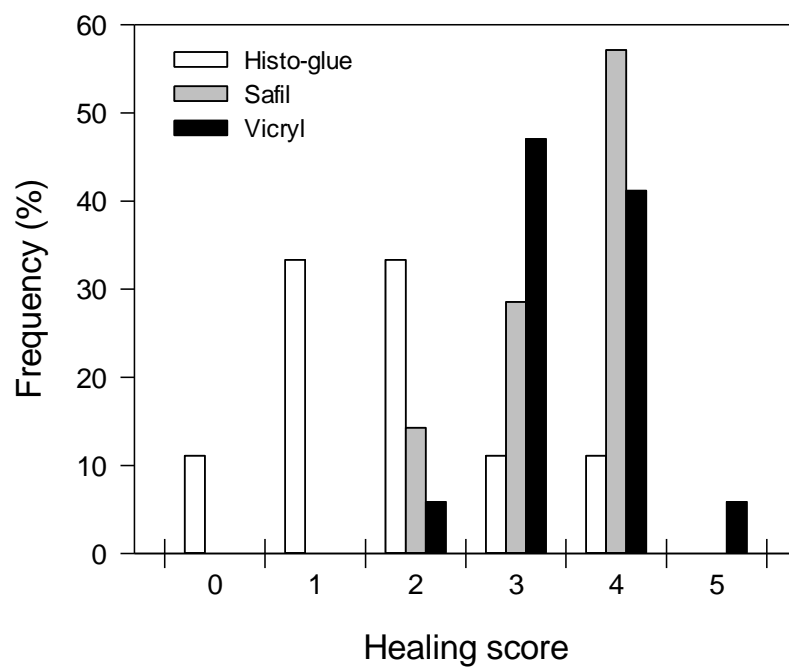


Figure 2. Healing of incisions. Lowest score indicates fully healed, healthy tissue, highest score is for gaping, infected, inflamed or badly healed incisions.